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# Gene Therapy Outcomes in Experimental Models of Inflammatory Arthritis

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## 1. Introduction

During the past decade the development of medical therapies for the treatment of the inflammatory arthropathies such as rheumatoid arthritis (RA) was revolutionized by the development of disease modifying anti-rheumatic biological drugs (DMARBDs) (Šenolt et al., 2009). DMARBDs were first discovered to be effective by altering intracellular signaling pathways and those gene expressional events that have proven to be relevant to RA pathogenesis and progression through the use of experimental studies in pre-clinical *in vitro* testing systems (Malemud, 2010; Malemud & Miller, 2008, Malemud & Pearlman, 2009; Marone et al., 2008; Walker et al., 2006). Those DMARBDs that appeared to have significant modifying effects on immune deregulation were then shown to ameliorate arthritis by virtue of their capacity to delay the onset or reducing the severity of inflammatory arthritis in several well-validated small animal models of RA (Feely et al., 2009). Finally, those DMARBDs which proved to have disease-modifying effects in rodent models of RA were then assessed for their efficacy, tolerability and safety in human RA clinical trials (Gibbons & Hyrich, 2009). Those successful DMARBDs are now fully incorporated into RA treatment regimens in the clinical practice setting.

## 2. Animal models of RA

Animal models of human RA have proven to be critical for determining the potential efficacy of DMARBDs for subsequent use in the treatment of human RA. The DMARBDs that are now employed for RA therapy were always first tested in RA animal models where the molecular events governing the onset and progression of RA disease activity could be rigorously and longitudinally assessed (Wooley, 2008; Leung et al., 2010). The DMARBDs that targeted pro-inflammatory cytokines relevant to RA progression such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1), IL-6, IL-12/IL-23 and IL-17 as well as other molecules whose activity correlated with progressive RA disease activity, such as CTLA-4 and CD20/CD-22 were shown to significantly modify the progression of inflammatory arthritis in mice (Finckh & Gabay, 2008).

### 3. The use of DMARBDs in RA

Despite improvement in the frequency of positive clinical responses in RA patients that have been obtained using DMARBDs when combined with immunosuppressive drugs such as methotrexate, the chronic use of DMARBDs in RA therapy has also revealed that serious adverse events typified by the onset of new infections, the development of refractoriness to DMARBDs as well as the long term specter of potential malignancies have also been documented (Salliot et al., 2009). These long-term treatment considerations have resulted in the need for a continuous stream of development of additional and hopefully novel RA therapies that specifically target molecules intimately involved in RA progression. One strategy that has been utilized for this purpose is the development of gene therapy for RA (Gaddy & Robbins, 2008; Jorgensen & Apparailly, 2010; Traister & Hirsh, 2008; Woods et al., 2008)

### 4. Gene therapy for RA

Over the past 7 years or so a considerable amount of research activity has been devoted to the development of gene therapeutic strategies that have resulted in 1) neutralization of the effects of IL-1 with an IL-1 receptor antagonist (IL-1-Ra) expression plasmid (Kim et al., 2003); 2) elevating the levels of Th2 cytokines exemplified by IL-10 (Traister & Hirsh, 2008), IL-4 (Kageyama et al., 2004) and IL-13 (Nabbe et al., 2005); 3) suppression of Th1 cytokines such as IL-18 (Smeets et al., 2003) and IL-17, the latter by modification of the indoleamine 2,3-dioxygenase pathway (Chen et al., 2011); 4) blunting of TNF- $\alpha$ -stimulated signaling (Denys et al., 2010) and interferon- $\beta$  activity (Adriaansen et al., 2006); 5) up-regulation of the protein inhibitor of activated STAT1 (PIAS) activity by stimulating the capacity of small ubiquitin-like modifier E3 ligase (SUMO E3) to alter inhibitor of  $\kappa$ B kinase $\alpha$  (IKK $\alpha$ ) phosphorylation (Liu & Shuai, 2008); 6) gene transfer of genetically modified chondrocytes into cartilage defects to promote cartilage regeneration (Steinert et al., 2008); and 7) promotion of adiponectin gene expression (Ebina et al., 2009). In addition, because new blood vessel formation is also intimately involved with perpetuating the chronic state of inflammation associated with RA, experimental gene therapy strategies designed to suppress the activity of pro-angiogenesis factors such as vascular endothelial growth factor (VEGF) (Afuwape et al., 2003) and Tie-2 (Chen et al., 2005) have also been earnestly pursued.

There also exists an elevated frequency of apoptotic cells in RA articular cartilage joints which is likely to contribute to diminished chondrocyte vitality (Malemud & Gillespie, 2005). By contrast, resistance to induction of apoptosis in RA synovial-like fibroblasts (Hutcheson & Perlman, 2008) and up-regulation of chemokines and adhesion molecules (Malemud & Reddy, 2008) are generally considered to be hallmarks of synovial tissue hyperplasia. Thus, apoptosis, chemokines and adhesion molecules may also be suitable targets for gene therapy strategies.

#### 4.1 Novel gene therapy strategies and improvements in the delivery of gene constructs

Several gene therapy strategies have taken advantage of recent advances in silencing RNA (siRNA) technology (Aigner, 2008; Blagbrough & Zara, 2009; Li & Huang, 2008; Lu & Woodle, 2008). Thus, siRNA has already been employed to generate tolerogenic dendritic

antigen-presenting cells (Khan et al., 2009; Zheng et al., 2010) or to “shut-down” pro-inflammatory cytokine production (Khoury et al., 2008). siRNAs have also been incorporated into a gene “cream” for delivery to inflamed synovial joints. This concept has already been used to regulate the biological activity of molecules such as osteopontin in a rodent model of RA (Takanashi et al., 2009).

Most importantly, vast improvements have occurred in developing new strategies for administering gene constructs. This has been achieved through the use of non-viral vectors (Adriaansen et al., 2006) adeno-associated viral (AAV) vectors (Tang et al., 2010) or lentiviral vectors (Gouze et al. 2002). In that regard, the development of useful vectors for preparing gene constructs has taken on greater significance in the field. Thus, these various approaches to delivering gene constructs of interest to synovial joints are likely to be critical because the improved administration of gene constructs and/or plasmids will be instrumental for future studies designed to regulate the activities of such RA-related molecules such as IL-17, IL-18, CD40/nuclear factor- $\kappa$ B, COT/Tpl-2 and extracellular matrix degrading proteinases to name just a few relevant targets for ameliorating inflammatory arthritis *in vivo* (Wisler et al., 2011).

The major emphasis in this chapter has been placed on systematically analyzing the recent advances since 2007-2008 when this field was last reviewed (Malemud, 2007; Woods et al., 2008; Dai et al., 2008). Thus, this review will focus its attention on several novel modalities for developing gene constructs as well as emerging strategies for delivering genes to inflamed synovial joints in the murine or rat Type II collagen-induced arthritis (CIA) model of RA (Wooley, 2008). This animal model of inflammatory arthritis is considered useful for the pre-clinical assessment of safety, tolerability and efficacy of gene constructs for their potential application to human RA.

## 5. Gene therapy in the CIA model

### 5.1 Pro-inflammatory and anti-inflammatory cytokines

The imbalance between pro-inflammatory cytokines such as IL-1, TNF- $\alpha$  and IL-6 and anti-inflammatory cytokines such as IL-4 and IL-10 have been shown to be a significant driving force in the progression of inflammation in animal models of arthritis and as such the gene targeting of these cytokines would be expected to alter the progress of arthritis severity (van de Loo & van den Berg, 2002). Thus, polarization of T-helper type 1 cells ( $T_h1$ ) to T-helper type 2 cells ( $T_h2$ ) in favor of  $T_h1$  appears to be at the root cause of immune-mediated dysfunction in RA (Asquith & McInnes, 2007). In RA,  $T_h1$ /  $T_h2$  polarization appears to account, at least in part, for the skewed repertoire of pro-inflammatory cytokines to anti-inflammatory cytokines. This change appears to be coupled to the deficient production and/or activity of  $T_h2$  cytokines, exemplified by IL-4 and IL-10, which may also account for accelerating the progression of RA. Additionally, the genesis of a specific T-cell subset, termed  $T_h17$ , also activates a key intracellular signaling pathway in RA, namely, JAK/STAT (Malemud, 2010). It has been postulated that activation of JAK/STAT signaling causes acute synovitis to progress into a chronic destructive arthritis (Khoury et al., 2008; Lubberts, 2008). It was not unexpected that gene therapy strategies employed in CIA have been directed at neutralizing the activity of IL-1, and/or TNF- $\alpha$  or towards augmenting the production of the anti-inflammatory cytokines, IL-4 and/or IL-10. Several of these studies have yielded some promising results.

There have been significant advances in suppressing the activity of TNF- $\alpha$  in several animal models of arthritis through targeted gene therapy. Thus, Adriaansen et al. (2007) showed

that a recombinant AAV5 vector (rAAV5) encoding the NF- $\kappa$ B-TNFR1-Ig construct reduced paw swelling in the rat arthritis model which was accompanied not only by decreased levels of TNF- $\alpha$ , but also by reduced levels of IL-1 $\beta$  and IL-6 whereas the level of IL-10 was increased. These results suggested that a coupling mechanism between TNF- $\alpha$  and IL-1/IL-6 must be pertinent to arthritis progression in CIA if suppression of TNF- $\alpha$  gene expression resulted in lower levels of IL-1 $\beta$ /IL-6 and elevated levels of IL-10 as well. Importantly, the rAAV5 vector remained localized to the injected joint and no trace of rAAV5 was found in the periphery. The suppressive effect of a rAAV5 vector encoding TR1 fused to the F<sub>c</sub> portion of mouse IgG1 (i.e. TNFRp55 extracellular domain-Ig) on inflammation was also seen in murine CIA (Khoury et al., 2007). In that study, there was a robust correlation between decreased levels of local TR1 and markers of inflammation that contributed to an overall reduction in the inflammation score.

Gould et al. (2007) employed a novel gene delivery system using plasmid vectors pGTLMIK and pGTTMIK from which luciferase and a dimeric TNF receptor were co-expressed in a doxycycline-regulated fashion, to show that administered GTTMIK delayed the onset of arthritis in murine CIA and the progression of inflammation as measured by reduced paw thickness. Additionally, the clinical score diminished but only when doxycycline was co-administered. The results of this study indicated that suppression of murine CIA could be achieved by inhibiting TNF- $\alpha$ -mediated events via gene expression-specific regulation. Inoue et al. (2009) used siRNAs which are double-stranded oligonucleotides that are designed to perfectly pair with the targeted mRNA in order to cause mRNA degradation to show that intra-articular delivery of TNF- $\alpha$  or IL1 $\beta$ -specific siRNAs reduced inflammation in rat CIA. The results of this study also showed that siRNA duplexes when administered *in vivo* to specifically degrade TNF- $\alpha$ , IL1 $\beta$ , IL-6 or receptor activator of nuclear factor  $\kappa$ B ligand (RANKL) mRNA also reduced the level of these cytokines *in vitro*. RANKL is a key growth factor in osteoclast differentiation and bone destruction in inflammatory arthritis (Choi et al., 2009).

Finally, Lai Kwan Lam et al. (2008) employed siRNA technology using a lentivirus-regulated B-cell activating factor (Lv-shBAFF) to limit BAFF levels. BAFF is a critical molecule in RA because it promotes the survival of activated B-cells (Townsend et al., 2010). In this study, Lv-shBAFF suppressed BAFF production. However, and unexpectedly so, silencing of BAFF gene expression also reduced the activity of T<sub>h</sub>17 cells as well as B-cell activity in murine CIA. The suppression of T<sub>h</sub>17 cell-mediated activity was accompanied by reduced synovial tissue inflammation. Moreover, Lv-shBAFF reduced the percentage of mice developing CIA by around 50% and reduced the onset time for arthritic pathology. Of note, computer tomography scanning failed to detect any narrowing of the joint space or changes in bone erosions when mice with CIA who had had Lv-shBAFF administered were compared to mice with CIA who were injected with an LV vector expressing shRNA for  $\beta$ -actin. In accompanying *in vitro* studies, Lv-shBAFF inhibited dendritic cell maturation which could have been responsible for the suppression of T<sub>h</sub>17 cell differentiation produced by Lv-shBAFF in CIA.

## 5.2 Signal transduction pathways

Gene targeting of the JAK/STAT and PI3K/Akt/PTEN/mTOR signal transduction pathways have been considered as an alternative to orally-administered enzyme inhibitors to suppress immune-mediated inflammation in animal models of arthritis (Camps et al.,



2005; Milici et al., 2008) as well as for inflammatory disorders in general (Ghigo et al., 2010). In that regard, Hildner et al. (2007) showed that targeting STAT4 by antisense phosphorothioate oligonucleotides reduced inflammation in murine CIA. In a correlative analysis (Hildner et al., 2007), STAT-4 deficient mice also developed significantly less severe arthritis than their wild-type counterparts. Further, T-cells recovered from the STAT-4 deficient mice produced less IL-6, TNF- $\alpha$  and IL-17. In another interesting study, adenoviral vectors encoding PTEN (AdPTEN) or  $\beta$ -galactosidase (AdLacZ) were injected directly into the joints of rats with CIA (Wang et al., 2008). AdPTEN was shown to reduce ankle circumference, articular cartilage degradation, radiographic and histology scores compared to rats with CIA injected with AdLacZ. AdPTEN also reduced microvessel density as well as VEGF and IL-1 $\beta$  levels while suppressing Akt activation. The suppressed activity of Akt correlated with a concomitant increase in the frequency of apoptotic cells in the arthritic ankle joints of rats with CIA. Taken together these results indicated that down-regulation of the PI3K/Akt/PTEN/mTor pathway by AdPTEN gene transfer could be an effective future strategy for not only suppressing the neoangiogenic response in synovial tissue that is a hallmark of joints with inflammatory arthritis (Szekanecz & Koch, 2008) but also as an alternative to using small molecule enzyme inhibitors in order to increase apoptosis in arthritic apoptosis-resistant synovial tissue (Hutcheson & Perlman, 2008).

### 5.3 T-cells, growth factors, metalloproteinase(s) and assorted other gene targets

T-cell receptor (TCR) gene transfer was originally proposed as a novel approach for use in antigen-specific immunotherapy (Fujio et al., 2006). Thus, in lupus-prone NZB/W F1 mice, nucleosome-specific TCRs and CTLA4-Ig-transduced cells suppressed autoantibody production and the development of nephritis whereas in CIA, TCRs recovered from T-cells that accumulated in the affected arthritic joints combined with TNFR-Ig-transduced cells or TCRs in combination with Forkhead box p3 (Foxp3) transcription factor-transduced cells, the latter a marker of the regulatory T-cell phenotype (Haque et al., 2010) suppressed the progression of arthritis and reduced bone erosions (Fujio et al., 2007a, 2007b). These results indicated that the production of antigen-specific T-cells engineered to express specific functional genes could be a productive immunotherapeutic strategy for future considerations in the treatment of human autoimmunity and arthritis.

Several growth factors pertinent to the abnormal increase in microvascular density that is a characteristic of RA synovial tissue have been targeted by gene constructs, including, VEGF (Afuwape et al., 2003), Ang-1 and Tie-2 (Fielder & Augustin, 2006). Because increased levels of synovial tissue Tie-2 and Ang-1 were shown to correlate with aberrant neoangiogenesis in CIA (DeBusk et al. 2003), Chen et al. (2005) studied the extent to which arthritis in CIA could be regulated by a Tie-2 gene construct. Thus, the severity of arthritis was markedly reduced when an adenovirus vector containing a soluble Tie-2 receptor (Ad.ExTek) was locally administered after the onset of arthritis. In addition, Ad.ExTek protected mice with CIA against bony erosions, suppressed neoangiogenesis, reduced the level of RANKL, but did not suppress anti-Type II collagen antibody production. However, a search of the PubMed literature since 2007 did not reveal any current studies directed at targeting angiogenesis via either Ang-1 or Tie-2 gene therapy suggesting that a greater focus has been placed on defining a common thread such as PI3K/Akt/PTEN/mTor pathway activation as a way of regulating neoangiogenesis in CIA (Wang et al., 2008).

The failure of chondrocytes to respond to insulin-like growth factor-1 (IGF-1) has been proposed as one of the mechanisms that underlie the inefficient repair of articular cartilage

in arthritis (van de Loo et al., 2008). Although the results of a study (Nixon et al., 2005) showed that synovial membrane co-transduced *in vitro* with an E1-deleted adenovirus vectors, one containing IGF-1 and the other IL-1 receptor antagonist (IL-1Ra) regulated by a cytomegalovirus promoter increased IGF-1 and IL-1Ra mRNA, an analysis as to the extent to which a gene strategy such as this one was capable of altering cartilage homeostasis in CIA was not performed. Thus, the more recent study results reported by Izal et al. (2008) becomes more significant because AAV-derived vectors containing IGF-1 increased secreted IGF-1 protein in cells infected with AAV-IGF-1 and increased cartilage protein synthesis 2 months after being locally administered into rats. However, this result could be not be reproduced in rats with CIA or in rats in which the arthritis was induced by mechanical injury indicating that AAV vectors capable of up-regulating the expression of IGF-1 *in vitro* was not an effective chondroprotective strategy.

Three additional targets for which gene therapy has been employed in experimental models of arthritis are worthy of comment. The down-regulation of MMP gene expression and inhibition of MMP activation are critical mechanisms for regulating cartilage and bone extracellular matrix degradation in RA (Zafarullah et al., 2008). Chia et al. (2008) employed the mouse model of CIA to show that the MMP-9 (92kDa gelatinase) gene was up-regulated in both the acute and chronic (late) stage of mouse CIA whereas elevated IL-1 $\beta$  gene expression was limited only to the acute stage of CIA. This result suggested that targeting MMP-9 by gene therapy could significantly limit the destruction of cartilage and bone extracellular matrix in CIA. Furthermore, because the activity of MMP-9 is regulated by neutrophil gelatinase-associated lipocalin (NGAL) which prevents the autocatalytic degradation of pro-MMP-9 (Gupta et al., 2007), NGAL synthesis could also be targeted by gene therapy to determine the extent to which down-regulating NGAL gene expression reduces the onset and/or severity of arthritis in CIA. Resistance to induction of apoptosis in RA synoviocytes results in perpetuating the inflammatory response in RA synovium for which several intracellular pathways that regulate the induction of apoptosis are known to converge contributing to RA pathogenesis and progression, including the development of pannus (Baier et al., 2003; Ospelt et al., 2004; Sweeney & Firestein, 2004; Malemud & Gillespie, 2005). Since it would be unlikely that inhibiting only one of these intracellular signaling pathways would induce apoptosis in RA synovium (Malemud, 2011), it was notable that Chen et al. (2009) showed that an E1B-55-kDa-deleted AV (Ad.GS1) driven by the human telomerase reverse transcriptase promoter induced cytolysis of human RA synovial fibroblasts and synoviocytes from rats with CIA *ex vivo*. Further, Ad.GS1 replicated only in arthritic rats where Ad.GS1 significantly reduced ankle size, the arthritis severity score, radiographic progression, and histological evidence of joint inflammation while also suppressing the expression of IL-1 $\beta$ , MMP-9 and prolyl-4-hydroxylase, the latter a marker of collagen biosynthesis (Kivirikko et al., 1989). Thus, Ad.GS1 was the first AV-vector driven by a telomerase promoter that was shown to ameliorate the severity of arthritis in CIA while also suppressing IL-1 $\beta$  and MMP-9, but with reduced collagen synthesis. The overall significance of Ad.GS1 in reducing prolyl-4-hydroxylase activity in RA cartilage and bone repair has not been completely elucidated. Finally,  $\alpha$ -1-antitrypsin (AAT) belongs to a class of acute phase reactant proteins which is also well known as an anti-inflammatory protein with marked tissue protective characteristics. Whereas serum levels of AAT are generally altered in RA, AAT levels failed to correlate with the titer of rheumatoid factor but AAT did correlate with erythrocyte sedimentation rate (Cylwik et al., 2010). Further, AAT levels were closely related to RA clinical activity as measured by the Disease Activity Score-28 criteria.

Recently, Grimstein et al. (2010; 2011) showed that mice with CIA treated either with AAT or rAAV-AAT alone or in combination with doxycycline delayed the onset and reduced the severity of arthritis. The severity of arthritis in CIA was correlated with reduced levels of BAFF and with lower anti-collagen autoantibodies suggesting that a major target of AAT therapy in CIA was to suppress the activity of hyperactive B-cells.

## 6. Conclusions

Significant progress has been made in the potential for future medical therapies of RA to employ gene therapy. In that regard, a few gene therapy strategies have already been tested in cell cultures and pre-clinical animal models of arthritis most notably in the CIA model in mice and rats. Of note, several targets for gene therapy such as IL-1Ra, TNF- $\alpha$ , and IL-17 have already yielded DMARDs or other forms of neutralizing compounds which are either currently being assessed in pre-clinical studies, tested in RA clinical trials or routinely used in the treatment of RA in clinical practice (Genovese et al., 2010; Finckh & Gabay, 2008; Gibbons & Hyrich, 2009; Ishiguro et al., 2011; S  nolt et al., 2009; Toh et al., 2010). However, the positive results obtained with various gene therapy strategies in cell cultures and/or in animal models of arthritis must always be cautiously interpreted as the previous supportive results with inhibitors of p38 kinase in arthritis animal models (B  hm et al., 2009; Hammaker & Firestein, 2010) which provided the impetus for the clinical testing of p38 kinase inhibitors proved to be disappointing in terms of clinical outcomes in human RA trials (Genovese, 2009). Thus, it remains to be seen if the biopharmaceutical industry will use pertinent targets for RA intervention such as IL-10, BAFF, MMP-9, etc. in gene therapy studies so that the effects of these gene constructs can be pre-clinically assessed. In addition, an increased attention to the potential negative biological effects of the viral vectors so commonly used to regulate the activity of these gene constructs must also take place. If no toxic or adverse effects occur in pre-clinical testing, then and only then should these gene constructs be further developed for their eventual assessment in RA.

## 7. Acknowledgments

Research in the Malemud laboratory is funded by Takeda Pharmaceuticals of North America and by Roche/Genentech. No further conflicts of interest relevant to this article were reported.

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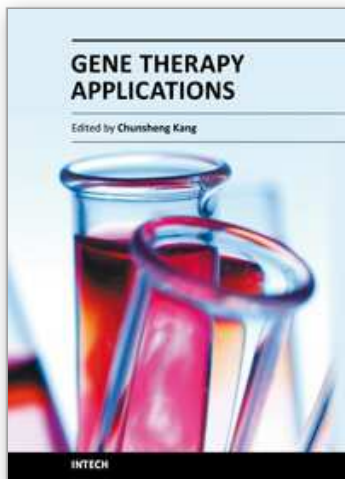
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## **Gene Therapy Applications**

Edited by Prof. Chunsheng Kang

ISBN 978-953-307-541-9

Hard cover, 492 pages

**Publisher** InTech

**Published online** 23, August, 2011

**Published in print edition** August, 2011

The aim of our book is to provide a detailed discussion of gene therapy application in human diseases. The book brings together major approaches: (1) Gene therapy in blood and vascular system, (2) Gene therapy in orthopedics, (3) Gene therapy in genitourinary system, (4) Gene therapy in other diseases. This source will make clinicians and researchers comfortable with the potential and problems of gene therapy application.

### **How to reference**

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Charles J. Malemud (2011). Gene Therapy Outcomes in Experimental Models of Inflammatory Arthritis, Gene Therapy Applications, Prof. Chunsheng Kang (Ed.), ISBN: 978-953-307-541-9, InTech, Available from: <http://www.intechopen.com/books/gene-therapy-applications/gene-therapy-outcomes-in-experimental-models-of-inflammatory-arthritis>

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